Investigating the role of DNA methyltransferases in the mantled somaclonal variation of oil palm

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DNA methyltransferase (DMTases) genes have emerged as targets of interest in the exploration of epigenetic mechanisms underlying the mantled variant phenotype in oil palm. Indeed, reduction in global DNA methylation rates and perturbations of floral phenotype could be due to underexpresion of these genes. Therefore, our research efforts focused on the isolation of members of all three families of DNA-methyltranferases identified in higher plants, namely MET, CMT (chromomethylase) and DRM (domain-rearranged). Using both a library screening and a PCR-based approach involving degenerated primers, we successfully cloned partial cDNA sequences belonging to all three families. The respective full-length cDNAs were subsequently obtained through the RACE (Rapid Amplification of cDNA Ends) method. For each DMTase type, we then determined the size of the family in the oil palm genome, and we examined the overall transcription level of each family member individually. The comparative transcription pattern of each class of DMTase was studied in normal and variant tissues through semi-quantitative Reverse-Transcription-PCR (sqRT-PCR) and Real Time quantitative PCR.
Our work shows for the first time the characterization of the three DNA methyltransferase gene families in oil palm, and addresses the hypothesis of their role in the determinism of the mantled variant phenotype.

Introduction

In plants and animals, DNA methylation has two essential roles: defending the genome against transposons and regulating gene expression. Plants use DNA methylation for genomic imprinting and to modulate the expression of transposable elements. In mammals, DNA methylation controls genomic imprinting, X-chromosome inactivation and the silencing of transposons. Mammalian DNA methylation is mostly restricted to symmetrical CG sequences. By contrast, plant DNA methylation occurs at CG, CNG (where N is any nucleotide) and CHH (an asymmetric site, where H is A, C or T) sequences, each of which has different genetic requirements for the maintenance of its methylation. The first aim of our work was to determine if oil palm contains the full complement of the DNA-methyltransferase types that are found both in Monocots and Dicots *e.g.* in model plants such as *Arabidopsis thaliana*.

In oil palm (*Elaeis guineensis* Jacq.), an average 5% of somatic embryo-derived somaclones have found to be affected by somaclonal variation, giving rise to an aberrant floral structure, male parts being transformed into carpel-like structures in flowers of both sexes (Rival and Parveez, 2005). Both genome-wide and sequence-specific DNA hypomethylation have been foound in variant tissues when compared to their normal counterparts (Jaligot et al, 2000, 2002 and 2004). It is thus of paramount importance to investigate relationship between changes in global methylation rates in 'mantled' oil palm variants and expression profiles of members of the three families of methyltansferases.

Oil palm encodes three families of plant DNA methyltransferases

In the model plant *Arabidopsis thaliana*, up to 10 DNA-methyltransferase sequences have been identified with one member from each family being predominantly expressed irrespective of tissue and developmental stage (Finnegan and Kovac, 2000). Moreover, dysfunctions of all three enzyme families have been involved in the formation of aberrant DNA methylation patterns, and, to varying extent, to the emergence of abnormal developmental phenotypes (Finnegan et al., 2000).

The complementarities between the three DNA-methyltransferase activities make them all likely candidates in our search for molecular markers of the "mantled" somaclonal variation. Our aim was to identify the respective role of each METase family in the hypomethylation of genomic DNA which has been measured in "mantled" material (Jaligot et al, 2000). The considerable sequence conservation detected between sequences identified in different plant species was used to implement two complementary homology-based approaches: i) a PCR-based strategy built upon the design of degenerated primers (Rose et al., 1998); ii) a screening of oil palm cDNA libraries.

Isolation and characterization of full length cDNA sequences for oil palm METase genes were primarily undertaken through the screening of cDNA libraries and data mining from our oil palm EST database (Jouannic et al, 2005). The Codehop approach (Rose et al, 2003) was also followed for the design of degenerated primers anchored to conserved motifs for the three studied families. The resulting sequences were then used in order to isolate the corresponding full-length coding sequences using the RACE (Rapid Amplification of cDNA Ends) technique.

MET1: The *MET1* gene family was isolated thanks to its extended homologies with plant and mammalian sequences (Finnegan and Dennis, 1993). MET1 has been identified very early as the main maintenance methyltransferase targeting symmetric sites (CG), although marginal *de novo* activity has been recently demonstrated on silenced transgene sequences. The *EgMET1* coding sequence is approx. 5 kb in length and is mostly similar to rice and maize sequences.

CMT3: The CMT (chromomethylase) family is unique to the plant kingdom (Henikoff and Comai, 1998); it is in charge of the maintenance of methylation at symmetrical sequence CpNpG. CMTs are characterized by the presence of a chromatin-association domain (or chromodomain) embedded in the catalytic part of the protein. CMT plays a major role in the long-term inactivation of repeated sequences such as retrotransposons. The *EgCMT* sequence (ca. 3 kb) displays extensive homologies with rice, maize and barley sequences.

DRM: The *de novo* methylation activity is controlled by the DRM (domain-rearranged) family, which shows an inversion in the arrangement of motifs in the catalytic domain, compared to the Dnmt3 *de novo* family found in mammalians (Finnegan and Kovac, 2000). This activity is required to establish methylation at previously unmethylated sites and to maintain methylation present at asymmetric (CNN) sites throughout mitotic divisions (Cao and Jacobsen, 2002). The *EgDRM* sequence (2,4 kb) is mostly similar to *DRM*-like sequences found in tobacco, rice and maize.

Global DNA hypomethylation in variant calluses is not consistent with changes in METases expression

Differential expression analyses by semi-quantitative RT-PCR and real-time quantitative PCR (rtQ-PCR) have been performed on oil palm embryogenic calli.

Repeatable overexpression of both *EgMET1* and *EgCMT3* genes was measured in Fast-Growing Calli (FGC, generating 100% of “mantled” palms) when compared to Nodular Compact Calli (which yield on average 5% of variant palms). The fate of *EgDRM* was found to be more variable: it was under expressed in variant material from some clonal lines and over expressed in some others.

We found a consistent over expression of both MET1 and CMT3 in FGC while the genome of FGC shows a 4.5% decrease in global methylation rate compared to that of NCC (Jaligot et al., 2000).

Our results parallel the major unresolved paradox in DNA methylation and cancer research (Laird and Jaenisch, 1994; Eads et al, 1999). Indeed, there is a net loss of m5C in many tumor cell genomes which is inconsistent with increased levels of transcripts from both *de novo* and maintenance DNA methyltransferases (James et al, 2003; Goll and Bestor, 2005). Our preliminary findings reveal a similar kind of relationship between DNA-METases expression and somaclonal variation in oil palm. They are presently being confirmed through qPCR analysis using different tissues and genotypes.

Conclusion

Full lengths cDNAs coding for three different DNA (cytosine-5)-methyltransferases families (namely MET, CMT and DRM) were isolated from oil palm (*Elaeis guineensis* L) and the corresponding genes designated as *EgMET*, *EgCMT* and *EgDRM*.

Global DNA hypomethylation which was previously measured in variant calluses is not related with decrease in expression of any of the three isolated METases

Directions for future research

Our result seem to show that global DNA methylation differences measured between normal and variant material could not be associated with parallel changes in DNA methyltransferases expression. There is evidence from both human cancer and plant DNA methylation research that methylation at a given locus occurs independently of the genome-wide methylation context. We are now focusing on DNA methylation around specifically targeted sequences, in relation with the "mantled" somaclonal variation. Indeed, the oil palm MADS orthologs of the B-type display differential transcription between normal and abnormal tissues (Adam et al., 2007a). Furthermore, recent collaborative work with MPOB has enabled the identification of a range of genes altered in their expression in abnormal tissue through the use of subtractive PCR (SSH) and subsequent macroarray hybridization (Beule et al, 2007)

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